

Regional Health Command-Central (RHC-C)  
Institutional Review Board

**HUMAN SUBJECTS RESEARCH  
PROTOCOL APPLICATION – Part B**

**1. PROTOCOL TITLE:** Clinical Evaluation of Decellularized Nerve Allograft with Autologous Bone Marrow Aspirate Concentrate (BMAC) to Improve Peripheral Nerve Repair and Functional Outcomes

**2. ABSTRACT:** This study is a prospective, multi-center, proof of principle, phase I human safety study (n=15) evaluating the sequential treatments of the Avance Nerve Graft, a commercially available decellularized processed peripheral nerve allograft, with autologous Bone Marrow Aspirate Concentrate (BMAC), a source of stem cells, for the repair of peripheral nerve injuries of up to 7 cm in length. The purpose of this study is to establish a knowledge product, evaluating the safety profile of the Avance Nerve Graft, followed by the application of BMAC to support further investment into the promising area of using stem cells in conjunction with scaffolds.

Each treatment separately is currently approved and used in the standard of care and has an established safety record with no reported serious adverse events. Avance Nerve Graft: (Cho, 2012) (Rinker, 2011) (Isaacs, 2013). BMAC: (Hendrich, 2009) (Jäger, 2009) (Centeno, 2016) (Hernigou, 2013). Subjects who have sustained a nerve conduction block injury in the upper extremity and require reconstruction of a nerve injury will be recruited and enrolled at three institutions. On day of surgery, the Avance Nerve Graft will be used at the site of nerve injury and then after completion of the first surgery, BMAC will be applied before closing the wound. BMAC from each patient will also be sent to Cleveland Clinic in order to confirm that the samples contain autologous bone marrow stem cells. Subjects will be followed post-surgery to evaluate the safety of the sequential treatments. While not powered to achieve statistical significance, due to the size limitation of a small safety study, secondary outcomes to evaluate an indication of efficacy of the two nerve repair treatments will be measured and compared to historic controls.

**3. OBJECTIVES/SPECIFIC AIMS/RESEARCH QUESTIONS.** The primary objective is to evaluate the safety of the Avance Nerve Graft procedure when followed by application of BMAC containing autologous stem cells as a proof of principle that allows for continued development of the concept of scaffolds and autologous stem cells. This clinical trial is not in support of any specific product or material development. It is intended solely as a knowledge product that supports the future investment in the use of scaffolds and autologous stem cells in regenerative medicine. The secondary objective is to measure the potential efficacy of the sequential treatments over 18 months as compared to historic controls.

The sequential use of Avance Nerve Graft and autologous BMAC will have an equivalent safety profile compared to each component individually and may demonstrate an improvement in the quality of nerve regeneration when compared to the current standard of care in a phase I clinical safety evaluation.

**Specific Aim 1:** Assess the safety profile of patients who sequentially receive Avance Nerve Graft followed by application of autologous BMAC for reconstruction of mixed peripheral nerve gaps up to 7 cm. The sequential treatment of the Avance Nerve Graft and BMAC will be compared to historic controls in regards to the number and severity of adverse events.

**Specific Aim 2:** Measure the efficacy of Avance Nerve Graft when followed by application of BMAC and compare levels of functional recovery to historic controls. Additional secondary efficacy endpoints such as: quality of life, extent of reinnervation, and correlation of short and long term outcomes will be measured. Percent recovery to baseline and time to recovery will be calculated based on analysis.

**4. MILITARY RELEVANCE** Military combat injuries to the extremities from blast, fragmentary, and ballistic injury can result in a spectrum of musculoskeletal trauma to include injuries to the soft tissues, vessels, nerves, and bone. This clinical safety evaluation may result in a knowledge product that provides confidence in the strategy that using a safe scaffold with a safe stem cell source is a safe approach. If successful this would provide validation to the military to continue investment in the area of regenerative medicine towards more complex solutions such as tissue regeneration and re-growing limbs. While not the primary goal of the study, secondary endpoints may indicate that this approach could lead to the treatment of longer nerve gaps, to better functional recovery, and return service members (as well as civilians) to their daily lives with increased functionality, diminished deficit, and ultimately a greater quality of life.

**5. BACKGROUND AND SIGNIFICANCE.** Traumatic injury and the associated peripheral nerve damage results in loss of controlled tissue animation, leaving the patient with severe functional disability. The defect in the injured nerve can be substantially more severe than the initial appearance as the zone of injury can necessitate extensive resection to obtain healthy nerve tissue. Additionally, the damaged distal stump undergoes time-dependent trophic and cellular changes, such as scarring, which degrade the stumps ability to support nerve regeneration (Lundborg, 2000). Deficits, which cannot be directly approximated, require reconstruction with a bridging material to serve as a conduit for the regenerating axons (Berger, 1978) (Noble, 1998) (Dvali, 2003) (IJpma, 2006). For years, the standard of care for peripheral nerve injuries (PNI) has been autologous nerve grafts, involving excision of a donor nerve from elsewhere on the patient's limbs and grafting it to the transected nerve site. However, these wounded service members have frequently sustained injuries to multiple limbs, often making autograft sites unavailable. Additionally, it follows that excision of nerve graft from a healthy limb will decrease said limb's nerve function and expected outcomes using autograft are underwhelming. A recent meta-analysis showed meaningful recovery rates for mixed nerve reconstructions to be a disappointing 60% (Brushart, 2011).

A myriad of synthetic scaffolds have been developed for use in lieu of autograft. However, commercially available nerve tubes have a limited functional length and recent clinical studies have found them to have very limited effectiveness in general clinical utilization (Wangensteen, 2010) (Lohmeyer, 2009). Undesirable handling characteristics can lead to adverse experiences for the patient, such as pain, soft tissue irritation and tube extrusion (Weber, 2000) (Rinker, 2011). These limitations have relegated their clinical application to non-critical sensory nerve defects and coaptation aides.

While the utilization of processed allogeneic tissues for the repair of tendon, skin, and bone injuries has been a mainstay for decades, the use of viable cellular nerve allograft was first introduced by Susan Mackinnon, M.D. in the 1990's. The results of her work were published in 2001 and detailed the outcomes in seven patients (Mackinnon, 2001). The results of this study showed promise, but adoption of cellular nerve allografts as a strategy for nerve reconstruction was hampered by the need for donor matched tissue and prolonged immunosuppression. Because of these limitations, an unprocessed cellular nerve allograft has shown limited clinical utility. The optimal scaffold upon which to place stem cells to improve nerve regeneration has been recognized as a processed decellularized nerve allograft. Only one such commercially available peripheral nerve graft exists, the Avance Nerve Graft. The Avance Nerve Graft represents an attractive alternative to harvesting autograft by offering numerous benefits to the patient, including decreased operative time and eliminating donor site morbidity. Furthermore, the efficacy and safety of this treatment option has already been established as equivalent to autograft.

**Avance Nerve Graft:** Introduced commercially in 2007, the Avance Nerve Graft is a decellularized, predegenerated, and sterilized extracellular matrix processed from donated human peripheral nerve tissue. The structure of the extracellular matrix is comprised of bundles of small diameter endoneurial tubes. The tissue is processed to remove cellular and non-cellular factors such as Schwann cells, fat, blood, axonal debris, and chondroitin sulfate proteoglycans, while preserving the three dimensional scaffold, vascular structure, and basal lamina structure of the nerve.

Regenerating axons can grow through the allograft scaffold, into the patient's distal nerve tissue toward the target muscle or skin. Unlike tube conduits, which rely on the host to form a rudimentary and provisional matrix within its hollow structures, the processed nerve allograft provides internal architecture inherent to nerve tissue. This internal structure is present upon implantation and ready to support regeneration (Graham, 2009) (Whitlock, 2009) (Neubauer, 2010). Avance Nerve Graft has an established track record for safety and efficacy (Brooks, 2012) (Cho, 2012) (Karabekmez, 2009) (Taras, 2013) (Guo, 2013) (Isaacs, 2013) (Lin, 2013) (Rinker, 2011). It has been used at a range of institutions; including small rural medical centers, level 1 trauma centers, academic medical centers, Veterans Administration medical centers, and military medical centers. Avance Nerve Graft has been utilized by General Surgeons, Plastic Surgeons, Orthopaedic Surgeons, Hand Surgeons, Neurosurgeons, Trauma Surgeons, Urologic Surgeons, Gynecologic Surgeons, Oral and Maxillofacial Surgeons, Neuro-otologists, Otolaryngologists, Podiatric and Oncologic Surgeons for the reconstruction of traumatic and iatrogenic peripheral nerve discontinuities (See Human Data below).

Comprehensive *in vitro* and *in vivo* biocompatibility evaluations provide support that Avance Nerve Graft is safe for use in humans. Efficacy studies in rat models have shown that, in terms of axon regeneration, Avance Nerve Graft is superior to a currently available conduit-style nerve guide (Whitlock, 2009) and compares favorably to isograft, which is similar to autograft (Graham, 2009). In a subsequent study examining nerve fiber density, it was found that the processed nerve allograft and isograft had nerve fibers evenly distributed across the cross section of the nerve. The processed nerve allograft density was superior to collagen nerve conduit, whose regeneration was found to be sparse and irregularly clustered throughout the cross section (Johnson, 2011).

Avance Nerve Graft has also been subjected to well established and accepted test panels for preclinical product safety evaluation and has been found to be non-toxic, non-reactive, non-sensitizing, non-pyrogenic, non-mutagenic, and non-irritant. Overall, studies utilizing well-established and validated animal models have demonstrated efficacy of the Avance Nerve Graft to reconstruct and repair peripheral nerve discontinuities and support axonal regeneration following transection injury.

**Scaffolds and Stem Cells Including Bone Marrow Aspirate Concentrate (BMAC):** Stem cells, when cultured together, do not self-organize into complex tissues due to the limits of transportation of nutrients and diffusion of waste products. Stem cells require scaffolds and 3-D matrices to grow into larger 3-D structures. The first notable example from the late 90's, the Vacanti Mouse, demonstrated the need for choosing the optimal scaffold and the optimal stem cell source. Since that demonstration, there has been an explosion in the types of scaffolds and stem cells proposed for use. The optimal scaffold will provide the requisite physical infrastructure upon which stem cells can engraft, transport materials, and rebuild new tissue.

This proposed clinical trial has the potential for demonstrating the safety of this proposed approach. Bone marrow represents the most universally accessible tissue for harvest of stem cells for a broad range of clinical applications. Bone marrow harvested by aspiration from the iliac crest has been shown to provide a rich, but variable, source of bone marrow stem cells which is then centrifuged in the surgery suite to create what is known as BMAC. Processing these cells involves "minimal manipulation" (without enzymatic digestion), an important factor in the FDA regulations. The Harvest Technologies system has been shown to achieve the highest number and concentration of progenitor cells after centrifugation (Hedge, 2014) and will be used in this study.

Experimental animal data has shown that the addition of stem cells to a nerve allograft scaffold increases the likelihood of successful regeneration and improves functional outcomes. Reported outcomes for stem cell seeded implants have demonstrated an increase in axonal growth in both conduits and allografts (Hu, 2007) (Dezawa, 2001) (Jackson, 2013) (Wang, 2010); increases in motor function (Hu, 2007) (Jackson, 2013) (Wang, 2010) (Chen, 2007); and superior assessment scores for sensory and mixed neurons (Kragh, 2012)

(Chen, 2007) (Mimura, 2004) (Reyes, 2001). If successful, this clinical evaluation will establish the safety of the proposed approach and justify further development in this material approach.

The Avance Nerve Graft and BMAC therapy are currently used separately in clinical practice as standard of care. Avance Nerve Graft has an established track record for safety and efficacy (Frykman, 1991) (Meek, 2005) (Cho, 2012) (Rinker, 2011) (Isaacs, 2013). BMAC has a clinically significant yield, does not induce immune-rejection, and requires minimal manipulation prior to usage. The use of autologous BMAC is considered a self-transplant and has a long track record of safety (Hendrich, 2009) (Jäger, 2009) (Centeno, 2016) (Hernigou, 2013). The following cited studies demonstrate that the Avance Nerve Graft and BMAC are safe when used separately in humans; large animal data is also provided in support of the two treatment modalities combined.

#### **Human Avance Safety Data:**

The following six studies are provided to reflect the published safety and efficacy profile of the Avance Nerve Graft. These studies showed no report of adverse events related to the use of Avance Nerve Graft and equivalence in efficacy when compared to autograft.

**Early clinical outcomes with the use of decellularized nerve allograft for repair of sensory defects within the hand.** (Karabekmez, 2009) The Mayo Clinic published their results regarding the performance of Avance Nerve Graft. Seven patients with ten nerve injuries were treated surgically. All subjects recovered near normal two point discrimination (2PD). **Relation to current study:** Avance Nerve Graft was demonstrated to be safe and effective with no reported signs of infection, rejection, or graft extrusion.

**Innovative treatment of peripheral nerve injuries: combined reconstructive concepts.** (Ducic, 2012) Ducic reported on outcomes from 54 discrete nerve repairs that were treated with various repair techniques. The authors found that Avance Nerve Graft returned functional improvements similar to those of other test groups. **Relation to current study:** No safety concerns were discussed and the treatment showed positive outcomes.

**Allograft reconstruction for digital nerve loss.** (Taras, 2013) Taras reported on an investigator initiated single center prospective study evaluating the clinical outcomes of digital nerve gaps in the hand measuring 30 mm or less repaired with Avance Nerve Graft. The study included 14 subjects with 18 nerve repairs. **Relation to current study:** No implant related adverse experiences were reported.

**Sensory outcomes after reconstruction of lingual and inferior alveolar nerve discontinuities using processed nerve allograft--a case series.** (Zuniga, 2015) Zuniga reported on an investigator initiated case series of Avance Nerve Graft for the reconstruction of lingual and inferior alveolar nerve discontinuities. The study included 26 subjects and 28 nerve repairs. Improvement in neurosensory function was reported in 87% of repairs. **Relation to current study:** There were no reported adverse experiences in this study.

**Processed nerve allografts for peripheral nerve reconstruction: a multicenter study of utilization and outcomes in sensory, mixed, and motor nerve reconstructions.** (Brooks, 2012) Brooks reported on the safety and efficacy of 132 individual nerve injuries treated with nerve allografts. The mean graft length was 22 ± 11 (5-50) mm. **Relation to current study:** No implant related adverse experiences were reported.

**A Multicenter, Retrospective Study of Avance® Nerve Graft Utilization, Evaluations and Outcomes in Peripheral Nerve Injury Repair (RANGER)** The RANGER Study is an AxoGen sponsored ongoing open label registry study designed to collect data about utilization, safety and functional outcomes in patients treated with the Avance Nerve Graft. There are 18 contributing centers designed to continuously monitor and collect injury, repair, safety and outcomes data for peripheral nerve injuries repaired with the Avance Nerve Graft,

nerve autograft and synthetic tubes. As of May 2015, the study included more than 650 nerve repairs enrolled across 18 centers and 40 surgeons (PRWeb, 2015). From the RANGER database, researchers Cho, Rinker, and Isaacs studied specific subgroups based on specific injuries or treatments. Cho reported on a subgroup of 71 nerves repaired with Avance Nerve Graft (Cho, 2012). Rinker reported on a subgroup of 24 subjects with 37 digital nerve repairs (Rinker, 2011). Isaacs report on a subgroup of repairs up to 50mm using Avance Nerve Graft in 13 subjects with 15 nerve repairs (Isaacs, 2013). All outcomes analysis in these studies demonstrated meaningful levels of sensory recovery and no implant related adverse experiences, further suggesting the safety of the Avance Nerve Graft in human subjects. **Relation to current study:** The RANGER clinical study was supported by AxoGen, Inc., the maker of the Avance Nerve Graft to further the understanding and outcomes of peripheral nerve repair. No safety concerns were discussed in the RANGER study at this time and no safety concerns were observed in the studies conducted by Cho, Rinker, and Isaacs.

#### **Human Bone Marrow Safety Data:**

The following four articles discuss the use of human bone marrow products in human subjects. None of the studies were associated with negative side effects and all showed positive outcomes with improved recovery associated with bone marrow treatment. These studies suggest that the use of bone marrow therapies like BMAC are safe and effective for use in human subjects.

**Safety of autologous bone marrow aspiration concentrate transplantation: initial experiences in 101 patients.** (Hendrich, 2009) **Summary:** This study evaluated the safety for the use of BMAC for new bone formation in 101 patients who suffer from various bone healing disorders. Patient recovery was observed 14 months after treatment where no complications in the form of infections, excessive new bone formation or renewed increase of complaints were noted. Subjectively, the bone marrow aspiration was not considered negatively by any of the patients. **Relation to current study:** This study demonstrates that the use of BMAC in humans has not shown signs of patient recovery complications and is safe for treatments that include BMAC application in conjunction with a scaffold product.

**Bone Marrow Concentrate: A Novel Strategy for Bone Defect Treatment.** (Jäger, 2009) **Summary:** This study evaluated the effects of BMAC applied to stimulate bone formation in patients with pseudarthrosis, bone cysts or revision endoprosthetic bone defects. The observed results for this specific treatment using BMAC had varied outcomes but none of the outcomes were associated with any significant or major complications or side effects. **Relation to current study:** The safety of an extracellular matrix with BMAC was evaluated and did not suggest any increased risk.

**A multi-center analysis of adverse events among two thousand, three hundred and seventy two adult patients undergoing adult autologous stem cell therapy for orthopaedic conditions.** (Centeno, 2016) **Summary:** This study followed recovery complications in 2372 orthopedic patients treated with stem cell injections for up to nine years. Within this treatment group with n=1590 (1949 injections) there were 114 adverse events, 7 of which were serious. This represents 7.2% of the group population reporting an AE, a small percentage of the study group. Bone marrow stem cells and MSC treatments have a lower rate of serious adverse events when compared to more invasive orthopedic procedures. **Relation to current study:** The results in Centeno, 2016 suggest that the utilization of bone marrow stem cells, MSC, or BMAC in methods as proposed in this study are safe for human use. The observed rate of AEs are lower than other treatments that are currently approved as safe for standard of care treatment.

**Cancer Risk Is Not Increased in Patients Treated for Orthopaedic Diseases with Autologous Bone Marrow Cell Concentrate.** (Hernigou, 2013) **Summary:** This study addressed the safety of using BMAC for the treatment of orthopedic lesions. The study specifically assessed the risk of cancer associated with this treatment. A total of 1873 patients were treated from 1990 to 2006 with BMAC cells and were monitored for cancer incidence from the date of the first operation (1990) until death, or until December 31, 2011. There was

no incidence of cancer formation at the site of treatment, and cancers that formed in other places in the body formed at the expected rate of cancer formation for the general population. **Relation to current study:** These results suggest that the use of BMAC for treatment is safe and does not increase the specific disease risk of cancer formation, suggesting that the use of BMAC in this study is safe and does not increase the risk of cancer formation.

### Large Animal Supporting Data:

The following seven large animal trials support the proposed use of peripheral nerve allografts combined with autologous bone marrow stem cells. Five of the seven animal models discussed below are primate models. These studies showed no increased incidence of AEs and suggest safety for the use in humans.

**Peripheral nerve defect repair with epineural tubes supported with bone marrow stromal cells: a preliminary report.** (Siemionow, 2011) **Summary:** This study was performed to assess the effects of nerve gap repair with epineural tubes filled with bone marrow stem cells as an alternative to autograft repair. Evidence of successful nerve regeneration was present in all animals at 6 weeks. All animals had full sensory recovery as at 12 weeks. Assessment did not reveal superiority of any group at 6 weeks post repair. **Relation to current study:** Bone marrow stem cells enhances nerve regeneration with increased myelination and neurotrophic factors when used in peripheral nerve repair when combined with a scaffold.

**Use of Tissue-Engineered Nerve Grafts Scaffold Included with Bone Marrow Mesenchymal Cells for Bridging 50-mm Dog Sciatic Nerve Gaps.** (Ding, 2010) **Summary:** This study evaluated a tissue-engineered nerve grafts consisting of a PLGA-based neural scaffold combined with autologous bone marrow stem cells for bridging 50-mm-long gaps in dog sciatic nerve. Results indicated that introduction of bone marrow stem cells to the scaffold promoted sciatic nerve regeneration and functional recovery. **Relation to current study:** This study showed that bone marrow stem cells plus nerve graft was better than graft alone and better than autograft, directly supporting the efficacy and safety of scaffolds used in combination with bone marrow stem cell products.

**Long-term outcome of the repair of 50 mm long median nerve defects in rhesus monkeys with marrow mesenchymal stem cells (MSC)-containing tissue engineered nerve grafts.** (Hu, 2013) **Summary:** This study evaluated the use of autologous bone marrow stem cells with a PLGA-based nerve grafts for bridging a 50-mm long median nerve defect in rhesus monkeys. At 12 months after grafting, the recovery of nerve function by graft/bone marrow stem cells was more efficient than that by scaffolds alone. In addition, this study demonstrated that graft/bone marrow stem cells could be safely used in the primate body. **Relation to current study:** The combination of bone marrow stem cells and a scaffold product were safely tolerated and effective in an animal model similar to humans.

**Bridging small-gap peripheral nerve defects using acellular nerve allograft implanted with autologous bone marrow stromal cells in primates.** (Wang, 2008) **Summary:** This study evaluated the effects of the transplantation of an acellular allogenic nerve graft combined with autologous bone marrow stem cells in a rhesus monkey in the radial nerve. The group treated with cultured bone marrow stem cells showed a statistically higher number of nerve fibers, conduction velocities and the peak amplitudes of action than those of the controls. **Relation to current study:** Direct validation of proposed study using acellular graft with bone marrow stem cells showing improvement over acellular allograft alone.

**Repair of extended peripheral nerve lesions in rhesus monkeys using acellular allogenic nerve grafts implanted with autologous mesenchymal stem cells.** (Hu, 2007) **Summary:** This study utilized acellular allogenic nerve segments implanted with bone marrow stem cells to repair a 40 mm defect in the rhesus monkey ulnar nerve. Results found that severely damaged ulnar nerves were structurally and functionally repaired within 6 months following placement of the MSC seeded allografts in all six animals studied. Findings

were the first demonstration of successful acellular allogenic nerve graft usage in primates. **Relation to current study:** Further support of safety and efficacy of the proposed approach in primates.

**Long-term observation of auto-cell transplantation in non-human primate reveals safety and efficiency of bone marrow stromal cell-derived Schwann cells in peripheral nerve regeneration.** (Wakao, 2010)

**Summary:** This study used a monkey peripheral nervous system injury model. No abnormalities were observed and suggest that auto-cell transplantation therapy is safe and effective for accelerating the regeneration of transected axons and for functional recovery of injured nerves. **Relation to current study:** Additional support for safety and use of bone marrow stem cells in peripheral nerve repair.

**Repairing large radial nerve defects by acellular nerve allografts seeded with autologous bone marrow stromal cells in a monkey model.** (Wang, 2010)

**Summary:** This study evaluated the potential of nerve grafts created from acellular allogenic nerve tissues combined with autologous bone marrow stem cells for repairing large peripheral nerve lesions in a rhesus monkey model. Bone marrow stem cells-laden allografts remarkably facilitated the recovery of the grasping functions of the animals, nerve conduction velocities, peak amplitudes of compound motor action potentials, greater axon growth, and higher target muscle weight. **Relation to current study:** Bone marrow stem cells plus nerve allografts were shown to be safe and efficacious in rhesus monkeys for large gap repair.

**6. RESEARCH DESIGN** This is a prospective, multi-center, phase 1 trial to evaluate the safety of Avance Nerve Graft and BMAC administered in sequential treatments and to measure the efficacy of the sequential treatments for peripheral nerve repair of the ulnar, median, radial, and musculocutaneous nerves of the upper extremities. The size of the nerve injury to be repaired will be limited up to 7 cm in length. To evaluate the safety of the sequential treatments, participants will be monitored on day 0 (day of surgery) and on day 10 (+/- 5 days) post-operative to track the number and severity of adverse events, if any, and to compare the adverse events to the results of previous studies that used Avance and BMAC treatments alone. To evaluate efficacy of the sequential treatments, participants will undergo standardized follow up assessments at 1 month, 6 month, 12 month, and 18 month post-operative to determine recovery of nerve function post repair as compared to historic controls for Avance Nerve Graft alone. We expect to establish a treatment modality that is both safe and that shows improvement with regard to efficacy without significant drawbacks of harvesting autograft.

**7. RESEARCH PLAN**

**7.1 Selection of Subjects**

**7.1.1. Subject Population.** Up to 15 male and females at each of three sites between the ages of 18 and 74 presenting with peripheral nerve injury in the upper limbs which after resection results in a nerve gap up to 7 cm, inclusive.

**Target Population:** The clinical safety evaluation of the sequential treatments of Avance Nerve Graft and BMAC will be applicable to both civilians and service members (active, retired, and reserves) with nerve injuries to their upper extremities.

**7.1.2. Source of Research Material.**

Source of Research Material	Clinical Purposes(Y/N)	Research Purposes (Y/N)
<i>Demographics</i>	Y	Y
<i>Medical History</i>	Y	Y

<i>Mechanism of Injury</i>	Y	Y
<i>Nerve Repair</i>	Y	Y
<i>Motor Domain</i>	Y	Y
<i>Sensory Domain</i>	Y	Y
<i>PROMIS assessment</i>	N	Y
<i>DASH assessment</i>	N	Y
<i>Rosen-Lundborg Study</i>	Y	Y
<i>Nerve Conduction Study</i>	Y	Y
<i>Bone Marrow Aspirate Concentrate (BMAC) sample</i>	N	Y
<i>Adverse Events</i>	Y	Y
<i>Concomitant Treatments</i>	Y	Y

### 7.1.3. Inclusion and Exclusion Criteria.

#### Inclusion Criteria

1. Male or non-pregnant female 18 to 74 years of age.
2. Undergoing peripheral nerve exploration or grafting with allograft in the upper extremity.
3. Subjects must be inpatients or scheduled for surgery at the time of study enrollment.
4. Has nerve conduction block injuries to the ulnar, median, radial or musculocutaneous nerve of either upper extremities that is less than two years from injury.
5. Be willing to undergo tension free end-to-end nerve graft coaptation on both the proximal and distal portion of the nerve gap with the Avance Nerve Graft.
6. Be willing to have bone marrow harvested from own body, concentrated, and applied to the site of nerve injury following the insertion of the Avance Nerve Graft.
7. Be willing to participate and able to comply with all aspects of the treatment and evaluation schedule over a 18-month duration.
8. Capable of giving their own consent to participate in the study, and willing to sign and date an IRB-approved written informed consent prior to initiation of any study procedures.
9. Nerve conduction injury affecting sensory and motor function or solely motor function in the upper extremity.
10. Nerve gaps following resection, up to 7Cm, inclusive.

#### Exclusion Criteria

1. Subjects with Type 1 Diabetes Mellitus or Type 2 Diabetes Mellitus requiring regular insulin therapy.
2. Subjects who are undergoing or expected to undergo treatment with chemotherapy, radiation therapy, or other known treatment which affects the growth of neural and/or vascular system.
3. History of neurodegenerative disease, neuropathy, or diabetic neuropathy.
4. History of chronic ischemic condition of the upper extremity.
5. Cognitive limitation or mental illness preventing informed consent.
6. Nerve injuries >2 years post initial injury.
7. Any participant who at the discretion of the Investigator is not suitable for inclusion in the study.

**7.1.4. Description of the Recruitment and Prescreening Process.** The treating physician, who may or may not be the study surgeon, will identify and refer potential study candidates to the research team by providing the patient's name and SSN or MRN. Under a partial waiver of HIPAA authorization, the research coordinators will



access and review the health information to assess whether the subject meets inclusion/exclusion criteria, and report back to the study surgeon. The study surgeon will then talk to the subject about the study and potential enrollment.

Medical Records used to verify inclusion/exclusion criteria for assessing initial eligibility of the trial will not be printed or stored. PHI/PII, including names and MRNs of all consented subjects referred by the treating physicians, will be kept on a Master file located in the orthopedic research team's locked office. The file linking study numbers with PII will be password protected. Access to this information will be limited to the orthopedic research team that is on the protocol.

**7.1.5. Subject Screening Procedures.** All patients between the ages of 18 and 74 admitted to the hospital or returning to clinic with an upper extremity injury with motor or sensory loss will be screened for eligibility at each site by the local Research Coordinator in close collaboration with treating physician who may refer the patient for study consideration and/or the study surgeon. Participants will be pre-screened according to the inclusion/exclusion criteria by the study team in accordance with the approved HIPAA waiver; however, informed consent must be obtained and documented prior to initiation of any procedures (such as physical exams, imaging, etc) that are performed solely for the purpose of determining eligibility for the research.

**7.1.6. Consent Process.** Informed consent will be obtained before beginning the final in-person screen. The conversation will be initiated by the Research Coordinator and investigator together. After reviewing all components of the informed consent form, the investigator and the Research Coordinator will answer any questions the patient has about participating in the study. The informed consent process will take place in a private setting and in the presence of a witness. Following completion of informed consent, the investigator will conduct a clinical assessment, medical history, and interview to confirm eligibility. Patients who are ineligible will not continue in the study and will be withdrawn.

All patients must be able to provide informed consent. After completion of informed consent, the participant information will be entered into the CRF where a subject ID will be assigned and final eligibility criteria confirmed. Recognizing the consent is an ongoing process, the research team will encourage the participants to ask additional questions that may arise during the course of their participation in the study. To encourage a high level of participation from eligible patients, the treating surgeon will be made aware of the research protocol and will be available to answer any questions his patient may have about enrollment in the study. Consent will be obtained in accordance with principles of GCP and ICH guidelines.

**7.1.7. Compensation for participation.** No compensation will be provided for participation.

## **7.2 Drugs, Dietary Supplements, Biologics, or Devices.**

### **7.2.1 N/A**

### **7.2.2**

## **Avance Nerve Graft**

Avance Nerve Graft is a human tissue for transplantation, processed and distributed in accordance with US FDA requirements for Human Cellular and Tissue-based Products (HCT/P) under 21 CFR Part 1271 regulations, US State regulations and the guidelines of the American Association of Tissue Banks (AATB). The graft is to be dispensed only by, or on the order of, a licensed physician. Indication For Use: The Avance Nerve Graft is processed nerve allograft (human) intended for surgical repair of peripheral nerve discontinuities to support regeneration across the defect.

## **Harvest Technologies Smart Prep 2 Centrifuge System**

The Harvest Technologies Smart Prep 2 is cleared by the FDA as a Class II 510K medical device: K052925. Indication For Use: This device is intended to be used in the clinical laboratory or intraoperatively at point-of-care for the safe and rapid preparation of a cell concentrate from bone marrow.

**7.3. Study Procedures/Research Interventions.** The intervention tested is the Avance Nerve Graft and BMAC treatments administered sequentially. The sequential treatments are not currently used together routinely at SAMMC. However, they are used routinely separately as needed. Surgical standard of care is to explore the nerve, identify and evaluate the defect or injury, and graft as indicated. Both treatments will be used sequentially at SAMMC for research purposes of the study.

The order of the intervention is as follows:

**1) Pre-Screening:**

The following is part of standard of care:

- Inclusion Criteria
- Exclusion Criteria
- Medical History

**2) Informed Consent**

**3) Pre-Operative Screening (Within 4 weeks of Operative Day):**

Once informed consent has been obtained, the subject will be assigned a subject ID and the following pre-operative procedures will be performed at this visit:

- Inclusion Criteria
- Exclusion Criteria
- Medical History (Number of units of blood donated pre-operatively)
- Demographics (gender, race, height, weight, history of smoking or tobacco use, alcohol use, co-morbidities)
- Nerve Injury History
- Vitals

**4) Operative Day Screening (Day 0):**

- Inclusion Criteria
- Exclusion Criteria
- Vitals

**5) Operative Day Treatments (Day 0):**

**Procedure 1:**

**Prepare Nerve Injury Site:**

Surgeons and other medical staff will prepare the site of nerve injury for surgery in accordance to standard surgical fashion.

**Complete Avance Nerve Graft Procedure:**

Medical staff specifically surgeons, who are properly trained and licensed will conduct and/or oversee the insertion of the Avance Nerve Graft. The Avance Nerve Graft will be inserted in the area of nerve injury according to the Instructions For Use in the package insert. See attached. Below is a picture of the Avance Nerve Graft that will be inserted in the nerve injury site (see Figure A).



**Figure A: Avance Nerve Graft**

**Steps Of How To Insert Nerve Graft:**

1. Determine the injury nerve diameter in millimeters using a suitable measuring instrument.
2. Select Avance Nerve Graft(s) of comparable diameter to match the native nerve and of sufficient length to ensure a tension free repair.
3. To prepare the Avance Nerve Graft:
  - a. Remove foil chevron pouch containing nerve graft, instructions for use, patient record labels, and Tissue Utilization Report (TUR) from the package.
  - b. Compare the distinct lot number on the foil chevron pouch with the lot number on the package. If the numbers do not match, DO NOT USE the product and notify AxoGen Customer Care immediately.
  - c. Using standard aseptic technique, peel open the outer foil chevron pouch and pass the inner chevron pouch to the sterile field for further handling.
  - d. Open the chevron pouch and remove the plastic tray.
  - e. Open the plastic tray and fill the pre-modeled thawing reservoir with room temperature sterile saline or sterile Lactated Ringer's solution. Do not heat the graft or add heated saline or Lactated Ringer's solution to the graft.
  - f. Allow Avance Nerve Graft to thaw completely before use which will take about 5 to 10 minutes. Once thawed, nerve graft is soft and pliable throughout. The nerve graft must be either implanted or discarded within 12 hours.
5. Handle nerve graft by outer most epineurium and avoid crimping or crushing the graft.
6. Implant nerve graft using the same tensionless surgical technique used when implanting a nerve autograft. Either end of the processed nerve allograft can be coapted to the proximal stump of the host nerve.
7. Destroy any thawed allograft tissue not used in the surgical procedure in accordance to procedures of disposing human tissue.
8. Complete and send the TUR back to AxoGen.

AxoGen, the company who created the Avance Nerve Graft, will provide training on the surgical implantation of the Avance Nerve Graft and how to complete the functional nerve assessments.

**Procedure 2:**

**Harvest Bone Marrow:**

In the operating room, after induction of anesthesia and prior to initiation of surgery, at a time when the patient would not feel pain, the surgeon(s) or other medical staff will use standard operating procedures to harvest bone marrow from the bone of the pelvis. General, spinal, local, or a combination will be used to provide anesthesia for the bone marrow aspiration from the pelvis. Bone marrow from the pelvis is available by aspiration through a single 3 mm incision.

A needle will be used to harvest between 40 to 60 ml of Bone Marrow Aspirate from the anterior or posterior iliac crest of the pelvis.

After aspiration, the needle is removed and a sterile bandage is applied similar to when blood is drawn. The total volume for all marrow samples will be up to 60 ml. The sterile bandage may be removed two days after surgery. The aspiration site is associated with minimal tenderness or pain, and is clinically comparable to percutaneous phlebotomy.

**Prepare Final BMAC:**

Trained medical staff will use the harvested 40- 60 ml of bone marrow aspirate and the BMAC system (comprised of the SmartPrep centrifuge and 60 ml BMAC kit, see figure B and C, respectively) to yield a final BMAC. The SmartPrep centrifuge and 60 ml BMAC kit will yield 7 to 10 ml of final BMAC for every 60 ml of aspirate. The centrifuge process takes about 15 minutes. For every ml of BMAC, there is a mean count of  $88 \pm 50 \times 10^6$  nucleated cells (Kevy and Jacobson, 2013) (Kevy and Jacobson, 2012) (Hegde, 2014) (Kevy and Jacobson 2014). Of the 7 to 10 ml of final BMAC that is yielded, half (3.5 to 5 ml) of the final concentrate, composed of over 100

million nucleated cells, will be injected on top of the Avance Nerve Graft following the Avance surgery. The second half (3.5 to 5 ml) of the final concentrate will be inserted into a sterile tube containing culture media and then properly shipped overnight to Cleveland Clinic for cell processing and colony assay to confirm that the BMAC indeed contains autologous bone marrow stem cells.



**Figure B:** SmartPrep 2 Centrifuge



**Figure C:** 60 ml BMAC kit

*Steps On How To Prepare Final BMAC:*

1. Remove processing disposable container from the packaging and place into holder apparatus.
2. Following aseptic technique, prep the red access site with a clean wipe.
3. Slowly dispense 60 ml of Bone Marrow Aspirate into marrowed chamber of processing disposable container through the red access site.
4. Place processing disposable container into SmartPrep centrifuge to process Bone Marrow Aspirate into BMAC. Make sure white access site on container aligns with white dot on rotor.
5. Place counterweight balance in rotor across from the disposable container.
6. Close lid. Press start button. Total process time is about 15 minutes. About 60 ml of aspirate will create 7 to 10 ml of final concentrate.
7. After cycle is complete, remove processing disposable container. Place container onto holder apparatus.
8. Following aseptic technique, prep the white access site with a clean wipe.
9. For cell resuspension, use the syringe with blood canula and yellow spacers to withdraw excess plasma from the plasma chamber by drawing up plasma until bubble are observed in the syringe.
10. Remove syringe and re-prep white access site.
11. To resuspend bone marrow cells into the remaining plasma, withdraw the remaining plasma into the 20 ml BMAC syringe with blood canula, but without spacers. Gently inject it back into the plasma chamber. Repeat step 2 to 3 times until cells are visibly resuspended in the plasma.
12. Then withdraw the total volume into the syringe. Observe the base of the plasma chamber to confirm all cells have been withdrawn into the syringe.
13. Transfer BMAC back into sterile field by connecting the BMAC syringe to the sterile luer-lok connector on a 20 ml sterile BMAC receiving syringe, which should be held by another medical staff member. Save the BMAC receiving syringe filled with final aspirate concentrate for BMAC application, which occurs after the insertion of the nerve graft.

Note: The 60 ml BMAC kit is for single-patient use only. Once, the final concentrate is obtained, the BMAC kit should be disposed as biohazardous waste. The kit (not including the centrifuge system) will be handled and disposed in accordance with standard regulations of the operating room.

Harvest Terumo (formerly Harvest Technologies), the company who supplies the BMAC system, will provide training to medical staff on how to use of the BMAC system and how to yield the final BMAC.

**Apply BMAC:**

Medical staff, specifically surgeons, who are properly trained and licensed will conduct and/or oversee the application of the final BMAC. Following the insertion of the nerve graft, of the 7 to 10 ml of final BMAC that is yielded using the BMAC system, half (3.5 to 5 ml) of the final concentrate will be withdrawn using a syringe,

which is then injected on top of the nerve graft. The other half of the concentrate (3.5 to 5 ml) of the final concentrate will be stored in a tube containing culture media and then properly shipped overnight to Cleveland Clinic for cell processing and colony assay. The research team will be responsible for shipping the contents to Cleveland Clinic.

## 6) Analysis of BMAC Samples

At Cleveland Clinic, the lab staff will only need half of final concentrate (3.5 to 5 ml) for each subject to collect data on the following variables:

- Cell Concentration,
- Connective Tissue Progenitor (CTP) Cells Prevalence
- CTP Concentration

Day 6 from when samples are cultured onto slides: Colony Forming Units (CFU) will be counted to calculate the mean performance for the following variables:

- Mean Cell Number
- Mean Cell Density
- Mean Cell Area Fraction
- Mean Area Fraction of Alkaline Phosphatase Expression

Analyzing the BMAC samples and collecting the data above will confirm that the aspirate contains bone marrow stem cells, commonly referred to as CTP cells.

All laboratory evaluations of BMAC slides will be conducted at Cleveland Clinic under the guidance of the consultants at that location. At Cleveland Clinic, BMAC samples will first be sterilely suspended in alpha-Minimal Essential Media complete media. Within 1 to 2 days of receiving the sample, the sample will be cultured onto a slide. A hemacytometer will be used to determine a nucleated cell count. Cells will be placed into a standardized assay for osteogenic CTPs (CTP-Os) (*four chambers on 2 Lab-tek slides*). On day 6 from when samples were cultured onto slides, the slides will be harvested using fixation and then stained with Alkaline phosphatase and DAPI, according to established protocols for colony assay analysis using Colonyze™ software.

BMAC samples will be coded and labeled with the subject's 2-digit ID number upon collection at the performance site. Subjects' names or other identifiers will not be shared with the collaborators at the Cleveland Clinic. Samples will be shipped to, stored, and secured in the Lerner Research Institute at Cleveland Clinic. Access to these samples will be limited to the consultants and supporting technicians at Cleveland Clinic. When samples are made into slides, the same coding numbers on the samples will be written on the slides. Any left-over aspirate concentrate from the sample will be discarded per Cleveland Clinic's policy.

The automated colony assay system will measure CTP prevalence and the proliferation, migration and differentiation of the CTP progeny.

## 7) Operative and Post-Operative Follow-Up Visits:

For the primary objective: Adverse events will be captured at Day 0 (day of surgery) and at Day 10 Post-operative (+/- 5 days).

For the secondary objective: Follow-up assessments will occur at Month 1 (+/- 2 weeks), Month 6 (+/- 2 weeks), Month 12 (+/- 2 weeks), and Month 18 (+/- 2 weeks) post-surgery. Assessments will be used to evaluate the subject's recovery of the targeted motor functional domain, sensory functional domain, and quality of life. Additional assessments will be used in order to compare results to historic controls and to determine the rate and level of reinnervation.

Study team personnel will conduct the post-operative follow up visits listed below after Day 0:

• **Day 0 (Day of Surgery):**

Operative Information:

- Surgery Information: Date of Surgery, Laterality of Site of Nerve Injury Repair
- Nerve Repair Procedures: Nerve Repair, Nerve Gap, Type of Repair, Suture Type, Suture Size, # of Sutures on Proximal End, # of Sutures on Distal End, Sealant Used, and Nerve Graft Wrapped, Distance from Proximal Nerve Stump to End Organ, Avance Nerve Graft Product Code, Quantity of Nerve Graft Used, Length of Implanted Nerve Graft, Duration of Surgical Procedure, Total Volume of Bone Marrow Aspirate Harvested, Total Volume of Final Aspirate Concentrate, Volume of Final Aspirate Concentrate Applied on Top of Avance Nerve Graft, Volume of Final Aspirate Concentrate Shipped to Cleveland Clinic.

Safety Evaluation:

- Evaluation of nerve repair site and iliac crest harvest site
- Assessment for any adverse events (number and level of severity)

Concomitant Treatments: Concomitant treatments will be captured to determine if the treatments are related to nerve injury/repair or related to an adverse event.

• **Day 10 ( $\pm$  5 days) Post-Operative:**

Safety Evaluation:

- Evaluation of nerve repair site and iliac crest harvest site
- Assessment for any adverse events (number and level of severity)

Concomitant Treatments (captured to determine if treatments are related to nerve injury/repair or related to an adverse event)

• **Month 1 (+/- 2 weeks) Post-Operative:**

Motor Domain Study- Effect limb and contralateral side (control)

- Range of Motion
- Muscle Strength Testing Grip Strength Testing
- Pinch Strength Testing

Sensory Domain Study- Effected limb and contralateral side (control): All sensory assessments will be conducted using the Neurosensory & Motor Testing System (NSMTS).

- 1 Point Static Discrimination
- 1 Point Moving Discrimination
- 2 Point Static Discrimination
- 2 Point Moving Discrimination

Rosen-Lundborg Study (compare results to historic controls)

- Sensory Domain (Innervation, Tactile Gnosis, and Finger Dexterity)
- Motor Domain (Innervation and Grip Strength)
- Pain/Discomfort Domain (Cold Intolerance and Hyperesthesia)

Quality of Life (Web-based)

- PROMIS Adult Self-Reported Physical Health Domain Assessment
  - Physical Function
  - Pain Intensity

- Pain Interference
- Fatigue
- Sleep Disturbance
- Pain Behavior
- Quick DASH Assessment

• **Month 6 ( $\pm$  2 weeks) Post-Operative:**

Motor Domain Study- Effect limb and contralateral side (control)

- Range of Motion
- Muscle Strength Testing
- Grip Strength Testing
- Pinch Strength Testing

Sensory Domain Study- Effected limb and contralateral side (control): All sensory assessments will be conducted using NSMTS.

- 1 Point Static Discrimination
- 1 Point Moving Discrimination
- 2 Point Static Discrimination
- 2 Point Moving Discrimination

Rosen-Lundborg Study (compare results to historic controls)

- Sensory Domain (Innervation, Tactile Gnosis, and Finger Dexterity)
- Motor Domain (Innervation and Grip Strength)
- Pain/Discomfort Domain (Cold Intolerance and Hyperesthesia)

Quality of Life (Web-based)

- PROMIS Adult Self-Reported Physical Health Domain Assessment
  - Physical Function
  - Pain Intensity
  - Pain Interference
  - Fatigue
  - Sleep Disturbance
  - Pain Behavior
- Quick DASH Assessment

• **Month 12 ( $\pm$  2 weeks) Post-Operative:**

Motor Domain Study- Effect limb and contralateral side (control)

- Range of Motion
- Muscle Strength Testing
- Grip Strength Testing
- Pinch Strength Testing

Sensory Domain Study- Effected limb and contralateral side (control): All sensory assessments will be conducted using NSMTS.

- 1 Point Static Discrimination
- 1 Point Moving Discrimination
- 2 Point Static Discrimination
- 2 Point Moving Discrimination

Rosen-Lundborg Study (compare results to historic controls)

- Sensory Domain (Innervation, Tactile Gnosis, and Finger Dexterity)
- Motor Domain (Innervation and Grip Strength)
- Pain/Discomfort Domain (Cold Intolerance and Hyperesthesia)

Quality of Life (Web-based)

- PROMIS Adult Self-Reported Physical Health Domain Assessment
  - Physical Function
  - Pain Intensity
  - Pain Interference
  - Fatigue
  - Sleep Disturbance
  - Pain Behavior
- Quick DASH Assessment

Nerve Conduction Study: Nerve conduction velocity (NCV) and Electromyography (EMG) testing will be conducted on the target muscle group to assess rate and level of motor and sensory reinnervation.

- Rate of Reinnervation (Motor and Sensory Domain)
- Level of Reinnervation (Motor and Sensory Domain)

• **Month 18 (± 2 weeks) Post-Operative:**

Motor Domain Study- Effect limb and contralateral side (control)

- Range of Motion
- Muscle Strength Testing
- Grip Strength Testing
- Pinch Strength Testing

Sensory Domain Study- Effected limb and contralateral side (control): All sensory assessments will be conducted using NSMTS.

- 1 Point Static Discrimination
- 1 Point Moving Discrimination
- 2 Point Static Discrimination
- 2 Point Moving Discrimination

Rosen-Lundborg Study (compare results to historic controls)

- Sensory Domain (Innervation, Tactile Gnosis, and Finger Dexterity)
- Motor Domain (Innervation and Grip Strength)
- Pain/Discomfort Domain (Cold Intolerance and Hyperesthesia)

Quality of Life (Web-based)

- PROMIS Adult Self-Reported Physical Health Domain Assessment
  - Physical Function
  - Pain Intensity
  - Pain Interference
  - Fatigue
  - Sleep Disturbance
  - Pain Behavior
- Quick DASH Assessment

Nerve Conduction Study: NCV and EMG testing will be conducted on the target muscle group to assess rate and level of motor and sensory reinnervation.



- Rate of Reinnervation (Motor and Sensory Domain)
- Level of Reinnervation (Motor and Sensory Domain)

**Timeline of Study**

Assessment							
Study Day / Visit	Pre-Operative (-4 wks to 0)	Surgery Day 0	F/U Day 10 (±5 days)	F/U Month 1 (±2 wks)	F/U Month 6 (±2 wks)	F/U Month 12 (± 2 wks)	F/U Month 18 (±2 wks)
Informed Consent	X						
Screening: Inclusion / Exclusion Criteria	X	X					
Demographics, Medical History & Nerve Injury History	X						
Operative Information		X					
Safety Evaluation of Adverse Events		X	X				
Concomitant Treatments		X	X				
Motor and Sensory Domain Studies				X	X	X	X
Rosen-Lundborg Study				X	X	X	X
PROMIS Assessment				X	X	X	X
Quick DASH Assessment				X	X	X	X
Nerve Conduction Study						X	X

**7.3.1 Collection of Human Biological Specimens.** Human biological (bone marrow aspirate concentrate) samples will be collected for this study.

Of the 7 to 10 ml of final BMAC that is yielded using the BMAC system, half (3.5 to 5 ml) of the final concentrate will be poured into a tube containing culture media and then properly shipped overnight to Cleveland Clinic for cell processing and colony assay.

BMAC samples will be coded and labeled with the subject's 2-digit ID number upon collection at performance site. Samples will be shipped to, stored, and secured in the Lerner Research Institute at Cleveland Clinic. Access to these samples will be limited to the consultants and supporting technicians at Cleveland Clinic. When samples are cultured into slides, the same coding numbers on the samples will be written on the slides. Any left over aspirate concentrate from the sample will be discarded per Cleveland Clinic's policy.

**Shipping Procedure for BMAC Samples:**

- 1) Place the labeled samples into an insulated shipping container (styrofoam box) with four freezer packs. Place Digi-temp temperature strip in box, next to sample, (Cole-Parmer, EW-09035-49).
- 2) Seal the shipping container with tape and affix the shipping label.
- 3) Record the shipping label information (written documentation of the number and date in the specimen tracking log). Also photograph the label to enable sharing with the Muschler Lab.
- 4) Promptly transport the shipping container for dispatch, (i.e. UPS or FedEx).
- 5) Notify the AI and/or supporting research team at Cleveland Clinic that the sample has been shipped. Provide the research team with the sample shipping and tracking information and photograph of the shipping label.

**7.3.1.1 Laboratory evaluations and special precautions.** BMAC samples will be collected and shipped to Cleveland Clinic Lerner Research Institute. Samples will be stored in a refrigerator up to 2 days, which then the samples are cultured onto slides and then discarded. After samples are made into slides, slides will be stored in slide box up to 8 days from when the sample was first received, which then the sample is processed for staining and colony analysis.

PI, AIs, and additional research team listed on the delegation logs will have access to the specimens, the clinical information, and linkage. After sample analysis, the samples will be discarded, following the Cleveland Clinic Lerner Research Institute policy guidelines for sample disposal. No specimens will be stored for future research.

The specimens will be analyzed at Cleveland Clinic to collect data on the following variables: cell concentration, CTP prevalence, and CTP concentration. CFUs will be counted on day 6 from when samples were plated into culture to calculate the mean performance for the following variables: cell number, density, cell area fraction, and area fraction of Alkaline Phosphatase expression.

**7.3.1.2 Specimen storage.** BMAC will be collected and shipped to Cleveland Clinic. After analysis, samples will be discarded, following proper Cleveland Clinic Lerner Research Institute policy guidelines for sample disposal.

- **Where and how specimens will be stored (including storage plan, etc.):**  
Samples will be stored in a refrigerator until ready for processing and analysis. After samples are made into slides, slides will be stored in slide box until they are processed for staining and colony analysis.
- **How specimens will be labeled:**  
All BMAC samples will be coded and labeled with the subject's 2-digit ID number upon collection at performance site. No personal identifiers will be used in the coding of human specimens.
- **Who will have the access to the specimens, the clinical information, and the linkage:** PI, AIs, and additional research team listed on the delegation logs will have access to the specimens, the clinical information, and linkage. After sample analysis is complete, the samples will be discarded at Cleveland Clinic, following their guidelines for sample disposal.
- **How specimens will be used (general and/or specific use):**  
The specimens will be analyzed at Cleveland Clinic to collect data on the following variables: cell concentration, CTP prevalence, and CTP concentration. CFUs will be counted on day 6 from when samples were plated into culture to calculate the mean performance for the following variables: cell number, density, cell area fraction, and area fraction of Alkaline Phosphatase expression.
- **Specify the length of time that specimens will be stored:** The samples will be stored up to 2 days until the samples are cultured onto slides and then discarded. The slide for each sample will be stored up to 8 days from when the sample was first received. No specimens will be stored for future research.

**7.3.2 Data Collection.** The data will be recorded on an approved paper Case Report Form (CRF). All data will be coded and then entered electronically via Research Electronic Data Capture (REDCap) which is a secure, web-based application designed exclusively to support data capture for research studies. The data collected in REDCap will be held as an electronic case report form (eCRF). Data collection from all sites will be overseen by the Clinical Manager.

All documentation supporting the CRF data, such as laboratory or hospital records, must be readily available to verify entries in the CRF. Documents (including laboratory reports, hospital records subsequent to Serious Adverse Events (SAEs), etc.) electronically transmitted will be de-identified and contain no patient identification information with the exception of a subject's assigned subject ID. This will help to ensure subject confidentiality.

Pre-Operative Information:

Demographics, Nerve Injury History, and Medical History will be collected on the CRF.

Operative Information:

Surgery Information: Date of Surgery, Laterality of Site of Nerve Injury Repair

Nerve Repair Procedures: Nerve Repair, Nerve Gap, Type of Repair, Suture Type, Suture Size, # of Sutures on Proximal End, # of Sutures on Distal End, Sealant Used, and Nerve Graft Wrapped, Distance from Proximal Nerve Stump to End Organ, Avance Nerve Graft Product Code, Quantity of Nerve Graft Used, Length of Implanted Nerve Graft, Duration of Surgical Procedure, Total Volume of Bone Marrow Aspirate Harvested, Total Volume of Final Aspirate Concentrate, Volume of Final Aspirate Concentrate Applied on Top of Avance Nerve Graft, Volume of Final Aspirate Concentrate Shipped to Cleveland Clinic.

Each site will be responsible for site monitoring consistent with ICH/FDA guidelines. Monitoring will include a combination of data checks in conjunction with the research monitor every 6 months. This will include reviewing the individual subject records, including consent forms, case report forms, supporting data, laboratory specimen records, any SAEs, unanticipated problems involving risk to subjects or others, and medical records (physicians' progress notes, nurses' notes, individuals' hospital charts), to ensure protection of study subjects, compliance with the protocol, and accuracy and completeness of records. The site monitor also will inspect sites' regulatory files to ensure those regulatory requirements are being followed. The overall Principal Investigator will interact with the research monitor at each site to review SAE reports, data checks and resolve safety issues if any should arise. The overall Principal Investigator and the site research monitor will determine the course of action necessary to meet safety goals and objectives. The overall Principal Investigator will monitor the safety of the data compiled across all sites.

**7.3.3. Human Biological Specimens/Tissue/Data Banking.** For Protocols Only Establishing a Repository, use Template P02R.

N/A

**Statistical Consideration**

**7.4 7.4.1 Sample Size Estimation.** Approximately 45 subjects total will be recruited total from the performance sites combined. Participants will be recruited at the time of peripheral nerve injury diagnosis. Up to 45 subjects will be enrolled to account for attrition.

**7.4.2 Primary (i.e., primary outcome variables) and secondary endpoints.**

**Primary Safety Endpoints:** The safety endpoints will be the comparison of the nature and incidence of AEs between the group of subjects receiving Avance with BMAC and the historical data of nerve repairs with Avance. Specifically, long-term study associated AEs, such as infection, wound dehiscence, neuropathy, carpal tunnel syndrome, bleeding, seroma, and lymphocele will be captured and analyzed together with any change in incidence of listed AEs which may be precipitated by treatment. AEs will be mapped to a MedDRA preferred term and system organ classification. The occurrence of the AEs will be summarized by repair type using MedDRA preferred terms, system organ classifications, and severity. All AEs will be listed for individual subjects showing both verbatim and preferred terms. Separate summaries of treatment-emergent SAEs and AEs related to repair will be generated.

**Secondary Efficacy Endpoint Comparing to Autograft: The secondary efficacy endpoint is based on the Rosen- Lundborg Scale total score and will be compared using an unpaired t-test.**

**Secondary Efficacy Endpoint Comparing to Avance Alone: The secondary efficacy endpoint is based on the functional recovery rates when using the Rosen- Lundborg Scale total score and will be compared using an unpaired t-test.**

### 7.4.3 Data analysis.

**Primary Safety Endpoints:** The safety endpoints will be the comparison of the nature and incidence of AEs between the group of subjects receiving Avance with BMAC and the historical data of nerve repairs with Avance. Specifically, long-term study associated AEs, such as infection, wound dehiscence, neuropathy, carpal tunnel syndrome, bleeding, seroma, and lymphocele will be captured and analyzed together with any change in incidence of listed AEs which may be precipitated by treatment. AEs will be mapped to a MedDRA preferred term and system organ classification. The occurrence of the AEs will be summarized by repair type using MedDRA preferred terms, system organ classifications, and severity. All AEs will be listed for individual subjects showing both verbatim and preferred terms. Separate summaries of treatment-emergent SAEs and AEs related to repair will be generated.

Any event reported on the CRF that occurs on or after the repair is defined as treatment-emergent. Additionally, it is assumed that an AE which was reported to have started on Day 0 without an associated onset time may have occurred after the repair. Hence, AEs occurring on Day 0 with no associated onset time are assumed to be treatment-emergent. Subject disposition will be presented for all subjects. Summaries will be broken down by visit as well as being presented over all visits. The number of subjects who completed the study and discontinued from the study will be provided. The reasons for early discontinuation also will be presented.

**Secondary Efficacy Endpoint Comparing to Autograft: The secondary efficacy endpoint is based on the Rosen- Lundborg Scale total score and will be compared using an unpaired t-test.**

Test of non-inferiority and superiority of Avance Nerve Graft to historical nerve autograft scores with respect to Rosen-Lundborg will be conducted using closed testing procedures. The hypotheses being tested are as follows:

$$H_{01}: \Delta \leq -\Delta_0 \quad \text{vs.} \quad H_{11}: \Delta > -\Delta_0$$

$$H_{02}: \Delta = 0 \quad \text{vs.} \quad H_{12}: \Delta \neq 0$$

where  $\Delta = \mu_C - \mu_A$  is the difference between the mean Rosen-Lundborg Scores for the Avance Nerve Graft & BMAC ( $\mu_A$ ) and the mean Rosen-Lundborg scores for the historical autograft controls ( $\mu_C$ ),  $\Delta_0$  is the non-inferiority margin 0.51. The null hypothesis of non-inferiority ( $H_{01}$ ) will be tested first and, if rejected, then the null hypothesis of superiority ( $H_{02}$ ) will be assessed. Given that the closed testing procedure is implemented, no adjustment for multiple testing will be required.

**Secondary Efficacy Endpoint Comparing to Avance Alone: The secondary efficacy endpoint is based on the functional recovery rates when using the Rosen- Lundborg Scale total score and will be compared using an unpaired t-test.**

Test of non-inferiority of Avance Nerve Graft plus BMAC to Avance Nerve Graft recovery rates with respect to Rosen-Lundborg scores will be conducted using closed testing procedures. The hypothesis being tested is as follows:

$$H_{01}: \pi_A - \pi_{AB} \geq \Delta \quad \text{vs.} \quad H_{11}: \pi_A - \pi_{AB} < \Delta$$

where  $\pi_A$  is the recovery of Avance Nerve Graft and  $\pi_{AB}$  is the recovery of Avance plus BMAC.  $\Delta$  is the non-inferiority margin 25%

Additional secondary endpoints for both comparisons will include:

- Percent recovery to baseline (defined as the difference in the measured assessment of the repaired nerve as compared with neighboring uninjured and/or contra-lateral side);
- Time to recovery;
- PROMIS Scores;
- DASH scores;

- Nerve Conduction Studies

**Baseline Comparability:** Baseline characteristics of the sites will be compared using the unpaired t-test or the Wilcoxon rank sum test for continuous variables, depending on their distributions. Percentage differences will be compared with the Fisher's exact test (or the  $\chi^2$  test, when appropriate). Additional secondary efficacy endpoints (each measure assessed separately) will be compared using non-parametric statistical tests (for instance a Kruskal-Wallis followed by corrected Wilcoxon rank sum tests as appropriate).

**Sharing study results across all sites:**

The site research coordinator will enter non-personally identifiable information into a central and secured web-based data management system known as REDCap. REDCap will link all data from each site. This data management system has incorporated state-of-the-art features for electronic data collection and is configured in accordance with best practices for information technology and research data management. Data collected at the Curtis Hand Center and Cleveland clinic will be communicated back to either SAMMC or WRNMMC by specific secure methods for compilation and analysis.

Upon completion of the study, the data management group and biostatisticians will generate the Tables, Forms, and Listings for the PI to review. Once verified and approved by the PI, this document will be finalized. Based on this study data, the PI will draft a study report. This report along with the Tables, Forms, and Listings will be provided to the collaborative investigators for review and edits. A copy will also be provided to the research team for review and comments.

Study results, including information that could potentially benefit the subject, will be made available to subject participants upon completion of the final study report. Certain results, such as screening results and improvement of function as recorded by follow-up assessments, will be shared with the subject during the study.

**BMAC Culture Analysis Outcomes:** Data from all colonies will be used to quantify and compare proliferation, migration (i.e., colony density), and differentiation (i.e., % of colonies expressing each marker, area fraction of colonies expressing each marker) among tissue types.

**Statistical Methods for BMAC Culture Analysis:** Descriptive statistics will be used to define the mean, 95% confidence interval, range, etc, for each parameter. The mean of each parameter will be compared between enrolled subjects and historical controls using Student's *t* test or Mann-Whitney nonparametric test. The distribution of each (untransformed) parameter will be described in terms of relative fit to a Gaussian distribution, skewness, evidence of multimodal distribution, etc. Data that follow a lognormal (skewed) distribution will be subjected to a natural-log transformation before each *t* test is performed, and data that cannot be adequately transformed will be analyzed using the Mann-Whitney nonparametric test.

**7.7 Confidentiality.** The research team will maintain the list linking the subjects' name and study number. This link will be stored separately from study data. The master list linking study numbers with personally identifiable information will be destroyed upon completion of the protocol and all data analysis. Anonymized data will be deleted from local site computers no later than five years after completion of the study. All hardcopy data and surveys will be stored in a locked research office cabinet with access only to the research team. All electronic data will be stored on a secure server with access only to research team for a period of no longer than 5 years following conclusion of the study. Research related documents (including electronic data) and medical records will be accessible only by key personnel specifically designated and authorized by the Principal Investigator. All such personnel will be properly trained and supervised regarding the management and handling of confidential materials. The Principal Investigator assumes full responsibility for such training, supervision, and conduct. This information will also be available for audit by study monitors and representatives of the local IRB, the MCC, the DOD, the FDA and the OHRP

**7.7.1 Certificate of Confidentiality. N/A**

**8.0 RISKS/BENEFITS ASSESSMENT**

**8.1 Risks:**

**General Operative Risks:**

- Infection-Rare (Event Rate < 1%)
- Blood loss-Rare (Event Rate < 1%)
- Anesthesia associated complications-Rare (Event Rate < 1%)
- Localized dermatitis-Rare (Event Rate < 1%)
- Drop in blood pressure-Rare (Event Rate < 1%)
- Syncope (fainting)-Rare (Event Rate < 1%)
- Bleeding at surgical site-Rare (Event Rate < 1%)
- Pain at surgical site-Likely (5% ≤ Event Rate < 10%)

**Complications Specific to Any Nerve Reconstruction Procedure:**

- Pain-Likely (5% ≤ Event Rate < 10%)
- Neuroma formation-Likely (5% ≤ Event Rate < 10%)
- Decreased or increased sensitivity-Likely (5% ≤ Event Rate < 10%)
- Impaired motor or sensory function-Likely (5% ≤ Event Rate < 10%)
- Risk that nerve may fail to regenerate-Likely (5% ≤ Event Rate < 10%)

**Avance Nerve Graft Risks:**

- Hypersensitivity, allergic reactions, or other adverse immune responses-Rare (Event Rate < 1%)
- Transmission of diseases of unknown etiology-Rare (Event Rate < 1%)
- Transmission of known infectious agents including, but not limited to viruses, bacteria, and fungi-Rare (Event Rate < 1%)

**Bone Marrow Extraction Risks:**

- Pain-Rare (Event Rate < 1%)
- Infection-Rare (Event Rate < 1%)
- Bruises-Rare (Event Rate < 1%)

**Risks of adding BMAC to Avance Nerve Graft**

- Increased immune response leading to nerve graft failure-Rare (Event Rate < 1%)

**Expected & Anticipated Adverse Events\*:**

- Mild incisional redness- Likely (5% ≤ Event Rate < 10%)
- Tenderness of surgical area- Likely (5% ≤ Event Rate < 10%)
- Mild edema of surgical area- Likely (5% ≤ Event Rate < 10%)
- Numbness- Likely (5% ≤ Event Rate < 10%)
- Pain at iliac crest harvest site- Likely (5% ≤ Event Rate < 10%)

**Risks of Nerve Conduction Studies:**

- Voltage of electrical pulses- very low

There is often some muscular ache or discomfort to an extremity during physical performance tests. All of your performance tests will be done under the supervision of trained study personnel to be sure the performance tests are done correctly.

\*These adverse events are considered expected and are not required to be recorded on the Adverse Events CRF unless they increase in severity. Expected adverse events, which are not serious are reported on the Continuing Review (CR) Progress Report. CR is generally performed on a 12-month cycle.

There is also a risk of breach of confidentiality of data belonging to the subjects enrolled in the study. Risk of breach of confidentiality will be minimized by keeping master list separate from data collection and consent forms. Subjects will be assigned a unique identification number, ID.

**8.2 Potential Benefits.** This study does not offer direct benefit from standard treatment to participants but is likely to yield important information about the safety of the sequential treatments of Avance Nerve Graft and BMAC containing autologous bone marrow stem cells in comparison to the treatment of Avance Nerve Graft alone.

The gold standard for nerve gap repair, autografting, carries significant patient risk and permanent morbidity to the donor site. Additionally, sources and quantities of autologous tissue for repairs are limited and, when faced with severe trauma, donor sites are often part of the zone of injury and simply not viable for harvest. The latter issue can be mitigated with allograft technology, and the use of Avance has shown promise for replacing the prevalence of autograft therapy, though improvements in gap repair speed and success rates are required for allografts to supplant autografts completely. This multicenter study will provide an evaluation of the safety and efficacy of combining Avance and autologous BMAC. Establishing a treatment modality that is non-inferior or superior to autograft and allograft alone will reduce procedure time, eliminate donor site morbidity, lower amputation rates, reduce long-term disability, promote earlier return to active duty/civilian employment, increase quality of life, and lower overall health care costs. The ultimate goal of this project is to provide the peripheral nerve injury patient with the safest and most efficacious treatment options.

## **9.0 ADVERSE EVENTS, UNANTICIPATED PROBLEMS, AND DEVIATIONS**

### **9.1 General Operative Risks:**

- Infection-Rare (Event Rate < 1%)
- Blood loss-Rare (Event Rate < 1%)
- Anesthesia associated complications-Rare (Event Rate < 1%)
- Localized dermatitis-Rare (Event Rate < 1%)
- Drop in blood pressure-Rare (Event Rate < 1%)
- Syncope (fainting)-Rare (Event Rate < 1%)
- Bleeding at surgical site-Rare (Event Rate < 1%)
- Pain at surgical site-Likely (5% ≤ Event Rate < 10%)

### **Complications Specific to Any Nerve Reconstruction Procedure:**

- Pain-Likely (5% ≤ Event Rate < 10%)
- Neuroma formation-Likely (5% ≤ Event Rate < 10%)
- Decreased or increased sensitivity-Likely (5% ≤ Event Rate < 10%)
- Impaired motor or sensory function-Likely (5% ≤ Event Rate < 10%)
- Risk that nerve may fail to regenerate-Likely (5% ≤ Event Rate < 10%)

### **Avance Nerve Graft Risks:**

- Hypersensitivity, allergic reactions, or other adverse immune responses-Rare (Event Rate < 1%)
- Transmission of diseases of unknown etiology-Rare (Event Rate < 1%)
- Transmission of known infectious agents including, but not limited to viruses, bacteria, and fungi-Rare (Event Rate < 1%)

### **Bone Marrow Extraction Risks:**

- Pain-Rare (Event Rate < 1%)
- Infection-Rare (Event Rate < 1%)
- Bruises-Rare (Event Rate < 1%)

### **Risks of adding BMAC to Avance Nerve Graft**

- Increased immune response leading to nerve graft failure-Rare (Event Rate < 1%)

### **Expected & Anticipated Adverse Events\*:**

- Mild incisional redness- Likely ( $5\% \leq \text{Event Rate} < 10\%$ )
- Tenderness of surgical area- Likely ( $5\% \leq \text{Event Rate} < 10\%$ )
- Mild edema of surgical area- Likely ( $5\% \leq \text{Event Rate} < 10\%$ )
- Numbness- Likely ( $5\% \leq \text{Event Rate} < 10\%$ )
- Pain at iliac crest harvest site- Likely ( $5\% \leq \text{Event Rate} < 10\%$ )

#### **Risks of Nerve Conduction Studies:**

- Voltage of electrical pulses- very low

There is often some pain and discomfort associated with post-surgical rehabilitation that is no greater than standard clinical care. All of your performance tests will be done under the supervision of trained study personnel to be sure the performance tests are done correctly.

\*These adverse events are considered expected and are not required to be recorded on the Adverse Events CRF unless they increase in severity. Expected adverse events, which are not serious are reported on the Continuing Review (CR) Progress Report. CR is generally performed on a 12-month cycle.

#### **9.2 Reporting Unanticipated Problems Involving Risks to Subjects or Others, Serious Adverse Events and Deaths to the Office of the IRB, RHC-C.**

All unanticipated problems involving risk to subjects or others, unexpected serious adverse events, and all subject deaths related or possibly related to the study will be reported promptly providing initial notification of the event as quickly as possible after the research team's knowledge of the event, but within five (5) business days of identification by phone (210-916-0606/2598), by e-mail (usarmy.jbsa.medcom-bamc.mbx.bamc-irb@mail.mil), by facsimile (210-916-1650) or via letter addressed to IRB Administrator, Regional Health Command-Central Office of the Institutional Review Board, Brooke Army Medical Center, Attn: MCHE-ZQ, Department of Quality and Safety, 3551 Roger Brooke Drive, Fort Sam Houston, TX 78234-6315. A complete written report will follow the initial notification within 10 working days.

#### **9.3 Research Monitor.**

The research monitor will review all unanticipated problems involving risk to subjects or others, serious adverse events and all subject deaths associated with the protocol and provide an unbiased written report of the event to the RHC-C IRB. Other responsibilities may be assigned by the IRB. The research monitor will comment on the outcomes of the event or problem and in the case of a serious adverse event or death comment on the relationship to participation in the study. The research monitor will also indicate whether he/she concurs with the details of the report provided by the study investigator. Reports for events determined by either the investigator or research monitor to be possibly or definitely related to participation and reports of events resulting in death will be promptly forwarded to the RHC-C IRB Office.

**10.0 WITHDRAWAL FROM STUDY PARTICIPATION.** Participants will be informed that they may discontinue the study at any time, for any reason. They will be assured that the medical care which they receive at the participating facility will not be affected should they elect to discontinue participation in the study.

**11.0 USAMRMC Volunteer Registry Database.** N/A

**12.0 REFERENCES.** Berger, A., and Millesi, H. (1978). Nerve grafting. Clin. Orthop. 49–55.

Brooks, D.N., Weber, R.V., Chao, J.D., Rinker, B.D., Zoldos, J., Robichaux, M.R., Ruggeri, S.B., Anderson, K.A., Bonatz, E.E., Wisotsky, S.M., et al. (2012). Processed nerve allografts for peripheral nerve reconstruction: a multicenter study of utilization and outcomes in sensory, mixed, and motor nerve reconstructions. Microsurgery 32, 1–14.

Brushart, T.M. (2011). Nerve Repair (Oxford University Press).



- Centeno, Christopher J. et al. "A Multi-Center Analysis of Adverse Events among Two Thousand, Three Hundred and Seventy Two Adult Patients Undergoing Adult Autologous Stem Cell Therapy for Orthopaedic Conditions." *International Orthopaedics* 40.8 (2016): 1755–1765.
- Center for Biologics Evaluation and Research, C. for D. and R.H. Cellular & Gene Therapy Guidances - Minimal Manipulation of Human Cells, Tissues, and Cellular and Tissue-Based Products: Draft Guidance.
- Chen, C.-J., Ou, Y.-C., Liao, S.-L., Chen, W.-Y., Chen, S.-Y., Wu, C.-W., Wang, C.-C., Wang, W.-Y., Huang, Y.-S., and Hsu, S.-H. (2007). Transplantation of bone marrow stromal cells for peripheral nerve repair. *Exp. Neurol.* 204, 443–453.
- Cho, M.S., Rinker, B.D., Weber, R.V., Chao, J.D., Ingari, J.V., Brooks, D., and Buncke, G.M. (2012). Functional outcome following nerve repair in the upper extremity using processed nerve allograft. *J. Hand Surg.* 37, 2340–2349.
- Dezawa, M., Takahashi, I., Esaki, M., Takano, M., and Sawada, H. (2001). Sciatic nerve regeneration in rats induced by transplantation of in vitro differentiated bone-marrow stromal cells. *Eur. J. Neurosci.* 14, 1771–1776.
- Ding, F., Wu, J., Yang, Y., Hu, W., Zhu, Q., Tang, X., Liu, J., and Gu, X. (2010). Use of tissue-engineered nerve grafts consisting of a chitosan/poly(lactic-co-glycolic acid)-based scaffold included with bone marrow mesenchymal cells for bridging 50-mm dog sciatic nerve gaps. *Tissue Eng. Part A* 16, 3779–3790.
- Ducic, I., Fu, R., & Iorio, M. L. (2012). Innovative treatment of peripheral nerve injuries: combined reconstructive concepts. *Annals of plastic surgery*, 68(2), 180-187.
- Referenced in Section 4 Human Data Ducic et al 2012
- Dvali, L., and Mackinnon, S. (2003). Nerve repair, grafting, and nerve transfers. *Clin. Plast. Surg.* 30, 203–221.
- Frykman, G., and Gramyk, K. (1991). Results of Nerve Grafting In: Gelberman R. Operative nerve repair and reconstruction. (JB Lippincott).
- Galanakos, S.P., Zoubos, A.B., Ignatiadis, I., Papakostas, I., Gerostathopoulos, N.E., and Soucacos, P.N. (2011). Repair of complete nerve lacerations at the forearm: an outcome study using Rosén-Lundborg protocol. *Microsurgery* 31, 253–262.
- Graham, James B., Xue, Qing-Shan, Neubauer, Debbie, and Muir, David (2009). A chondroitinase-treated, decellularized nerve allograft compares favorably to the cellular isograft in rat peripheral nerve repair. 2, 19–29.
- Guo, Y., Chen, G., Tian, G., and Tapia, C. (2013). Sensory recovery following decellularized nerve allograft transplantation for digital nerve repair. *J. Plast. Surg. Hand Surg.* 47, 451–453.
- Hegde, V., Shonuga, O., Ellis, S., Fragomen, A., Kennedy, J., Kudryashov, V., & Lane, J. M. (2014). A prospective comparison of 3 approved systems for autologous bone marrow concentration demonstrated nonequivalency in progenitor cell number and concentration. *Journal of orthopaedic trauma*, 28(10), 591-598.
- Hendrich, Christian et al. "Safety of Autologous Bone Marrow Aspiration Concentration Transplantation: Initial Experiences in 101 Patients." *Orthopedic Reviews.* 1.2 (2009): e32. *PubMed*.
- Hernigou, Philippe et al. "Cancer Risk Is Not Increased in Patients Treated for Orthopaedic Diseases with Autologous Bone Marrow Cell Concentrate." *J Bone Joint Surg Am* 95.24 (2013): 2215–2221. *jbjs.org*.
- Hu, N., Wu, H., Xue, C., Gong, Y., Wu, J., Xiao, Z., Yang, Y., Ding, F., and Gu, X. (2013). Long-term outcome of the repair of 50 mm long median nerve defects in rhesus monkeys with marrow mesenchymal stem cells-containing, chitosan-based tissue engineered nerve grafts. *Biomaterials* 34, 100–111.
- Hu, J., Zhu, Q.-T., Liu, X.-L., Xu, Y., and Zhu, J.-K. (2007). Repair of extended peripheral nerve lesions in rhesus monkeys using acellular allogenic nerve grafts implanted with autologous mesenchymal stem cells. *Exp. Neurol.* 204, 658–666.

- Ijpma, F.F.A., Nicolai, J.-P.A., and Meek, M.F. (2006). Sural nerve donor-site morbidity: thirty-four years of follow-up. *Ann. Plast. Surg.* 57, 391–395.
- Isaacs, J. (2013). Major peripheral nerve injuries. *Hand Clin.* 29, 371–382.
- Jackson, W.M., Alexander, P.G., Bulken-Hoover, J.D., Vogler, J.A., Ji, Y., McKay, P., Nesti, L.J., and Tuan, R.S. (2013). Mesenchymal progenitor cells derived from traumatized muscle enhance neurite growth. *J. Tissue Eng. Regen. Med.* 7, 443–451.
- Jäger, M., Jelinek, E., Wess, K., Scharfstädt, A., Jacobson, M., Kevy, S., Krauspe, R. (2009). Bone Marrow Concentrate: A Novel Strategy for Bone Defect Treatment. *Current Stem Cell Research & Therapy.* 4, 34–43.
- Johnson, P.J., Newton, P., Hunter, D.A., and Mackinnon, S.E. (2011). Nerve endoneurial microstructure facilitates uniform distribution of regenerative fibers: a post hoc comparison of midgraft nerve fiber densities. *J. Reconstr. Microsurg.* 27, 83–90.
- Karabekmez, F.E., Duymaz, A., and Moran, S.L. (2009). Early clinical outcomes with the use of decellularized nerve allograft for repair of sensory defects within the hand. *Hand N. Y.* N 4, 245–249.
- Kragh, Kirby, J.M., and Ficke, J.R. *Combat casualty care : lessons learned from OEF and OIF. Chapter 9: Extremity Injury.* (2012) Editor-in-chief, Martha K. Lenhart; medical editor, Eric Savitsky; military editor, Brian Eastridge. Pgs. 393–484.
- Lin, M.Y., Manzano, G., and Gupta, R. (2013). Nerve allografts and conduits in peripheral nerve repair. *Hand Clin.* 29, 331–348.
- Lohmeyer, J.A., Siemers, F., Machens, H.-G., and Mailänder, P. (2009). The clinical use of artificial nerve conduits for digital nerve repair: a prospective cohort study and literature review. *J. Reconstr. Microsurg.* 25, 55–61.
- Lundborg, G. (2000). A 25-year perspective of peripheral nerve surgery: evolving neuroscientific concepts and clinical significance. *J. Hand Surg.* 25, 391–414.
- Lundborg, G., and Rosén, B. (2004). The two-point discrimination test—time for a re-appraisal? *J. Hand Surg. Edinb. Scotl.* 29, 418–422.
- Mackinnon, S.E., Doolabh, V.B., Novak, C.B., and Trulock, E.P. (2001). Clinical outcome following nerve allograft transplantation. *Plast. Reconstr. Surg.* 107, 1419–1429.
- Meek, M.F., Coert, J.H., and Robinson, P.H. (2005). Poor results after nerve grafting in the upper extremity: Quo vadis? *Microsurgery* 25, 396–402.
- Mimura, T., Dezawa, M., Kanno, H., Sawada, H., and Yamamoto, I. (2004). Peripheral nerve regeneration by transplantation of bone marrow stromal cell-derived Schwann cells in adult rats. *J. Neurosurg.* 101, 806–812.
- Neubauer, D., Graham, J.B., and Muir, D. (2010). Nerve grafts with various sensory and motor fiber compositions are equally effective for the repair of a mixed nerve defect. *Exp. Neurol.* 223, 203–206.
- Noble, J., Munro, C.A., Prasad, V.S., and Midha, R. (1998). Analysis of upper and lower extremity peripheral nerve injuries in a population of patients with multiple injuries. *J. Trauma* 45, 116–122.
- PRWeb. (2015). AxoGen Inc.'s Avance Nerve Graft Data Presented During Bese Clinical Papers Session at the 70<sup>th</sup> Annual Meeting of the American Society for Surgery of the Hand. Vocus, Inc. Sep. 10, 2015.
- Reyes, M., and Verfaillie, C.M. (2001). Characterization of multipotent adult progenitor cells, a subpopulation of mesenchymal stem cells. *Ann. N. Y. Acad. Sci.* 938, 231–233; discussion 233–235.

- Rinker, B., and Liao, J.Y. (2011). A prospective randomized study comparing woven polyglycolic acid and autogenous vein conduits for reconstruction of digital nerve gaps. *J. Hand Surg.* 36, 775–781.
- Siemionow, M., Duggan, W., Brzezicki, G., Klimczak, A., Grykien, C., Gatherwright, J., and Nair, D. (2011). Peripheral nerve defect repair with epineural tubes supported with bone marrow stromal cells: a preliminary report. *Ann. Plast. Surg.* 67, 73–84.
- Taras, J.S., Amin, N., Patel, N., and McCabe, L.A. (2013). Allograft reconstruction for digital nerve loss. *J. Hand Surg.* 38, 1965–1971.
- Wakao, S., Hayashi, T., Kitada, M., Kohama, M., Matsue, D., Teramoto, N., Ose, T., Itokazu, Y., Koshino, K., Watabe, H., et al. (2010). Long-term observation of auto-cell transplantation in non-human primate reveals safety and efficiency of bone marrow stromal cell-derived Schwann cells in peripheral nerve regeneration. *Exp. Neurol.* 223, 537–547.
- Wang, D., Liu, X.-L., Zhu, J.-K., Jiang, L., Hu, J., Zhang, Y., Yang, L.-M., Wang, H.-G., and Yi, J.-H. (2008). Bridging small-gap peripheral nerve defects using acellular nerve allograft implanted with autologous bone marrow stromal cells in primates. *Brain Res.* 1188, 44–53.
- Wang, D., Liu, X.-L., Zhu, J.-K., Hu, J., Jiang, L., Zhang, Y., Yang, L.-M., Wang, H.-G., Zhu, Q.-T., Yi, J.-H., et al. (2010). Repairing large radial nerve defects by acellular nerve allografts seeded with autologous bone marrow stromal cells in a monkey model. *J. Neurotrauma* 27, 1935–1943.
- Wangensteen, K.J., and Kalliainen, L.K. (2010). Collagen tube conduits in peripheral nerve repair: a retrospective analysis. *Hand N. Y. N* 5, 273–277.
- Weber, R.A., Breidenbach, W.C., Brown, R.E., Jabaley, M.E., and Mass, D.P. (2000). A randomized prospective study of polyglycolic acid conduits for digital nerve reconstruction in humans. *Plast. Reconstr. Surg.* 106, 1036–1045; discussion 1046–1048.
- Whitlock, E.L., Tuffaha, S.H., Luciano, J.P., Yan, Y., Hunter, D.A., Magill, C.K., Moore, A.M., Tong, A.Y., Mackinnon, S.E., and Borschel, G.H. (2009). Processed allografts and type I collagen conduits for repair of peripheral nerve gaps. *Muscle Nerve* 39, 787–799.
- Zuniga, JR. (2015). *Sensory outcomes after reconstruction of lingual and inferior alveolar nerve discontinuities using processed nerve allograft--a case series.* *Journal of Oral Maxillofacial Surgery.* 73, 734-44.

**13.0 TIME REQUIRED TO COMPLETE THE RESEARCH (including data analysis).** The study is anticipated to require 60 months from start of enrollment to be completed, including data analysis. Patients will be enrolled at time of PNI diagnosis and followed at day 10, 1 month, 6 month, 12 month and 18 month.

Data collection from all sites will be overseen and verified for accuracy by the Clinical Manager. De-identified data will be shared between performance sites.

**14.0 STUDY CLOSURE PROCEDURES** Protocol Closure Report will be submitted at the end of the study. Hard copy documents containing subject data and patient identifiers (and contact information) will be stored in secure document containers (file cabinets, lockers, drawers, etc.) accessible only to research personnel. The data entered in REDCap will downloaded as a compiled data file be kept as electronic records and hard copy signed consent forms will be kept in a locked filing cabinet for 6 years following study completion for future audit purposes. The research team will maintain the list linking the subjects' name and study number. This link will be stored separately from study data. The master list linking study numbers with personally identifiable information will be destroyed upon completion of the protocol and all data analysis. Anonymized data will be deleted from local site computers no later than five years after completion of the study. The signed informed consent documents and HIPPA authorizations will be retained locally for 3 and 6 years respectively.